F16. 14

	1/3/)	40	•
50	99	13		179 191
GTCCGGGCCCATGAGGCGACGAAGGAGGAGGAGAAAAAAAA	CGGCTTTTTACCCAGCCCGGACTTCCGAGGGCCGGCCCGAGGGCCCGAGGGCCCGAATGGCACGAGGCCGAACGAGGCCGAACGAGGCCGAACGAGGCCGAACGAGGCCGAACGAGGCCGAACGAACGAACGAACGAACGAACGAACGAACGAACGAACGAACGAACGAACGAACGAACGAACGAACGAACAAC			TGAGGATGAGCTGGAGGAGGGGGTCAGTTAAA CGAGGCGAGCCAGA GCTCAGCCCCG
18	51	100	114	147 166
p70a.hum p70b.hum	p70a.hum p70b.hum	p70a.hum p70b.hum	p70a.hum p70b.hum	p70a.hum p70b.hum
	18 GTCCGGGCCCATGAGGCGACGAAGGAGGGGGA 34 AGAGAGAGAGAGAGAGAGAGAGAGAGAGA	GTCCGGGCCCATGAGGCGACGAAGGAGAGGAGGAGAS AGAGAGAGAGAGAGAGAGAGA	GECCCATGAGGGGAGAGAGAGAGGAGGAGGGGGAAGAGAGAG	GTCCGGGCCCATGAGGCGACGAAGGAGAGAGAGGGGGAA AGAGAGAGAGA

NIDOTITUTE CUEET (DIN E 26)

F16. 1E

p70a.hum p70b.hum	180	TGAAAGCATGGACCATGGGGGAGTT GGAC 208 CGGACGCATGTCCCCTTGCCGAGTTGAGGGCAG 224
p70a.hum p70b.hum	209	CATATGAACTTGGCATGGAACATTGTGAGAAAT 241 CTGGCCTAGAGCCTGTGGGACACTATGAAGAGG 257
p70a.hum p70b.hum	242	TTGAAATCTCAGAAACTAGTGTGAACAGAGGC 274 TGGAGCTGAGACCAGCGTGAACGTTGGCC 2905
p70a.hum p70b.hum	275 291	CAGAAAAATCAGACCAGAATGTTTTGAGCTAC 307 CAGAGCGCATCGGGCCCCACTTGCTTTGAGCTGC 323
p70a.hum p70b.hum	308	TTCGGGTACTTGGTAAAGGGGGCTATGGAAAGG 340 TGCGTGTGGGCAAGGGGGGCTATGGCAAGG 356
p70a.hum p70b.hum	341	TTTTTCAAGTACGAAAAGTAACAGGAAGCAATA 373 TGTTCCAGGTGCGAAAGGTGCAAGGCACCAACT 389

PCT/US99/17595

		3/3	n		
406	439 455	472 488 2/3	505	538 554	571 587
CTGGGAAATATTTGCCATGAAGGTGCTTAAAAA TGGGCAAAATATATGCCATGAAAGTCCTAAGGA	AGGCAATGATAGTAAGAATGCTAAAGATACAGAGGGCAAGAGGACACAG	CTCATACAAAAGCAGAACGGAATATTCTGGAGGCACACACA	AAGTAAAGCATCCCTTCATCGTGGATTTAATTT CAGTGAAGCACCCCTTTATTGTGGAACTGGCCT	ATGCCTTICAGACTGGTGGAAACTCTACCTCA ATGCCTTCCAGACTGGTGGCAAACTCTACCTCA	TCCTTGAGTATCTCAGTGGAGGAGAGTTTATTATTATTCCTTGAGTGGCGAGCTCTTCA
374 390	407	440 456	473	506 522	539 555
p70a.hum p70b.hum	p70a.hum p70b.hum	p70a.hum p70b.hum	p70a.hum p70b.hum	p70a.hum p70b.hum	p70a.hum p70b.hum

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PCT/US99/17595

p70a.hum p70b.hum p70a.hum p70b.hum p70a.hum	572 588 605 621 638 654	TGCAGTTAGAAAGAGAGGAATATTTATGAAAG 604 CGCATCTGGAGGCATCTTCCTGGAAG 620 ACACTGCCTGCTTTTACTTGGCAGAATCTCCA 637 ATACGGCCTGCTTTTACTTGGCAGAATCACGC 653 TGGCTTTGGGGCATTTACATCACAGGGATCA 670 TGGCCTGGGCATCTCAAAAGGGGATCA 6865
p70a.hum p70b.hum	671	TCTACAGAGACCTGAAGCCGGAGAATATCATGC 703 TCTACCGGGACCTCAAGCCCGAGAACATGC 719
p70a.hum p70b.hum	704	TTAATCAGGGCATGATGAACTAACAGACT 736 TCAGCAGGGCCACATCAACTGACCGACT 752
p70a.hum	737	TTGGACTATGCAAAGAATCTATTCATGATGGAA 769 TTGGACTCTGCAAGGAGTCTATCCATGAGGGCG 785

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F16. 11

CAGTCACACACATTTTGTGGAACAATAGAAT 802	ACATGGCCCCTGAAATCTTGATGAGAAGTGGCC 835	ACAATCGTGCTGTGGATTGGTGGAGTTGGGAG 86882	OATTAATGTATGACATGCTGACTGGAGCACCCC 3015 CCCTGATGTACGACATGCTCACTGGATGGCGGC 317	2 CATTCACTGGGGAGAATAGAAAGAAAACAATTG 934	5 ACAAAATCCTCAAATGTAAACTCAATTTGCTC 967
CCGTCACTCACACCTTCTGCGGCACCATTGAGT 818	ACATGGCCCCTGAGATTCTGGTGCGCAGTGGCC 851	ACAACCGGGCTGTGGACTGGTGGAGCTTGGGGG 884		8 CCTTTACCGCAGAAACCGGAAGAAAACCATGG 950	1 ATAAGATCATCAGGGCAAGCTGGCACTGCCC 983
770	803	836 852	869	902	935
p70a.hum	p70a.hum	p70a.hum	p70a.hum	p70a.hum	p70a.hum
p70b.hum	p70b.hum	p70b.hum	p70b.hum	p70b.hum	p70b.hum

F16. 11

	CCTACCTCACACAGAAGCCAGAGATCTGCTTA 1000	AAAAGCTGCTGAAAAGAATGCTGCTTCTCGTC 1033	TGGGAGCTGGTCCTGGGGACGCTGGAGAAGTTC 1066	AAGACTCATCCATTCTTTAGACACATTAACTGGG 1099	AAGAACTTCTGGCTCGAAAGGTGGAGCCCCCT 1132	TTAAACCTCTGTTGCAATCTGAAGAGGAGTTAA 1165
	CCTACCTCACCCAGATGCCCGGGAACCTTGTCA 1016	AAAAGTTTCTGAAACGGAATCCCAGCAGCGGA 1049	TTGGGGGTGGCCCAGGGGATGCTGCTGATGTGC 1082	AGAGACATCCCTTTTTCCGGCACATGAATTGGG 1115	ACGACCTTCTGGCGTGTGGACCCCCTT 1148	TCAGGCCCTGTCTGCAGTCAGAGGAGGACGTGA 1181
70a.hum 70b.hum 70a.hum 70b.hum 70b.hum 70b.hum 70b.hum	968 984	1001	1034	1067	1100	1133
	p70a.hum	p70a.hum	p70a.hum	p70a.hum	p70a.hum	p70a.hum 1133
	p70b.hum	p70b.hum	p70b.hum	p70b.hum	p70b.hum	n70h hum 1149

PCT/US99/17595

CAACTCTCAGTG 1231	GGGTTTTACAT 1264		A ATCCGATCAC 1330	CGAACACCTG 1363
CAGCCCTCAGCG 1247	GGGCTTCACAT 1280		3 CTGCGCTCAC 1346	CGGGTCCCCG 1379
C A A C T C T C A G T G C A G C C C T C A G C G			A ATCCGATCAC SCTGCGCTCAC	CGAACACCTG
CTGTCGACAGCCCAGATGACT	A A A B C C A A T C A G G T C T T T C T A G A G A G C C A A C C A G G C C T T C C T	ATGTGGCTCCATCTGTACTTGA	AAAAATTTTCCTTTGAACCAAAATCCGATCAC	CT CGA AGA TTTATT T GGC AGC C C ACGA A C ACCTG
CGGTGGACAGTCCTGATGACA		ACGTGGCCGTCTGTCCTGGA	AGGGCTTCTTCCAGCCCAAGCTGCGCTCAC	CC AGG CGC CTC A A C AGT A G C C C C G G G T C C C C
p70a.hum 1199	p70a.hum 1232	p70a.hum 1265	p70a.hum 1298	p70a.hum 1331
p70b.hum 1215	p70b.hum 1248	p70b.hum 1281	p70b.hum 1314	p70b.hum 1347
			p70a.hum 1199 CTGTCGACAGTCCTGATGACTCAACTCTCAGTG p70b.hum 1232 AAAGTGCCAATCAGTCTTTCTGGTTTTACAT p70a.hum 1248 AGAGTGCCAATCAGGTCTTTCTGGGTTTTACAT p70a.hum 1265 ATGTGCTCCAACCAGCCTTTCTGGGTTTTCACAT p70b.hum 1265 ATGTGGCTCCATCTGTACTTGAAAGGAGAAGGGGCTTCAAAGG	

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FIG. I

1392	1425 1445	1458 1478	1491 1511	1524 1541	1557
TCAGCCCAGTCAAATTTTCTCCT GGGGAT TCAGCCCCTCAAGTTCTCCCCTTTGAGGGG	TTCTGGGGAAGATGCTTCGGCCAGCACAGCA	AATCCTCAGACACCTGTGGAATACCCAATGGAAAAGCTACCTAC	ACAAGTGGCATAGAGCAGATGGATGTGAATG CGCCCTCGACCACGCCCTCTCCCCATCCGTC	AGTGGGAAGCATCGGCACCACTTCCAATACGA CCCCCTCAGGGA CCAAGAAGTCCAAGAGGG	CAGCGAACTCTGGGCCATACAAAAAGCTGGGTGCTGCCGTGGGGGTCCAGGGCGCTAGGGAAGCCGGGT
. 1364	. 1393	. 1426 . 1446	. 1459	. 1492	. 1525
p70a.hum 1364 p70b.hum 1380	p70a.hum 1393 p70b.hum 1413	p70a.hum 1426 p70b.hum 1446	p70a.hum 1459 p70b.hum 1479	p70a.hum 1492 p70b.hum 1512	p70a.hum 1525 p70b.hum 1542

OS76EESG OSESO1

1590	1623 1640	1656 1673	1706	1722 1738	1753
TTTCCCATGATCTCCAAACGGCCAGAGCACCTG	CGT ATGAATCTATGACAGAGCAATGCTTTTAAT	GAATTTAAGGCAAAAAGGTGGAGAGGGAGATGT	GTGAGCATCCTGCAAGGTGAAACAAGACTCAAA	A TGACAGTT TCAGAGATCAA TGTCAT TACATA	GAACACTTCGGACAC - AGGAAAAATAAACGGTG
GGGGGTGAGGGTAGCCCTTGAGCCCTGTCCCTG	CGGCTGTGAGAGCAGGACCCTGGGCCAGTT	CCAGAAACCTGGGGGTGTGTGTCTGGGGGGTGGGGGT	GTGAGTGCGTATGAAAGTGTGTGTCTGCTGGGG	CAG-CTGTGCCCTGAATCATGGGCACGGAGG	
p70a.hum 1558	p70a.hum 1591	p70a.hum 1624	p70a.hum 1657	p70a.hum 1690	p70a.hum 1723
p70b.hum 1575	p70b.hum 1608	p70b.hum 1641	p70b.hum 1674	p70b.hum 1707	p70b.hum 1739
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GAAGA

CTGATGTGT

GCTCTGAATCACTGTGAGT

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F16.1

1786 1804	1819	1 8 25 1 0/3 0	1885	1918
GATTTTAAAAAATCAATTCAATGGTGCAAAAAA 1786 TGGAAGATTAAAGGGCTGAATCATGAAAAA 1804	AACTTAAAGCAAAATAGTATTGCTGAACTCTTA 1819 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	GGCACATCAATTGATTCCTCGCGACATCT	TTCTCAACCTTATCAAGGATTTTCATGTTGATG 1885	ACTCGAAACTGACAGTATTAAGGGTAGGATGTT
p70a.hum 1754 p70b.hum 1772	p70a.hum 1787 p70b.hum 1805	p70a.hum 1820	p70a.hum 1853	p70a.hum 1886
<u>'d</u>	ja ja	ď	ď	α.

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p70a.hum... 2117

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2017	2050	11/30 88 05/11	2116
CTATAATACTTGCAACTAAGGACAAATTAGCA	GCAAGCTTGGTCAACTTTCCCAGGCAAAT	GGAAGGCAAAGACAAAGAACTTACCAATTG/	TGTTTTACGTGCAAACAACCTGAATCTTTTT 2116
p70a.hum 1985	p70a.hum 2018	p70a.hum 2051	p70a.hum 2084
	CTATAATACTTGCAACTAAGGACAAATTAGCAT	CTATAATACTTGCAACTAAGGACAAATTAGCAT GCAAGCTTGGTCAAACTTTTCCCAGGCAAATG	CTAT GCAA GGAA

2346

TCAGIAACCCAGCTGCGGAGCCT

ATT

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p70a.hum... 2315

2314

T G G

TGTGGCTCGTTTGAGGGATTGGGGTGGACC

p70a.hum... 2282

F.G. =

ATTCAGCTCATTATGAAAACATCCCAAACTTT 2182	AAAATGCGAAATTATTGGTTGGTGTGAAGAAG 2215	CCAGACAACTTCTGTTTCTTCTTGGTGAAAT 2248	AATAAATGCAAATGAATCATTGTTAACACAGC 2281
p70a.hum 2150	p70a.hum 2183	p70a.hum 2216	p70a.hum 2249

F16. 2A-

RRRDGFYPAPDFRHREAEDMAGVFDIDLDMARGRRARGAGAAMAAVFDLDEMARGRRARGAGAAMAAVFDLDE	OPEDAGSEDELEEGGGLNESMDHGGVGFTELGM 85 TEEGSEGEGEPELSPADACPLAELRAAGLE-PV 55	EHCEKFEISETSVNRGPEKIRPECFELLRVLGK 99 GHYEEVELTETSVNVGPERIGPHCFELLRVLGK 885	13	NAKDTAHTKAERNILEEVKHPFIVDLIYAFQTG 165 NAKDTAHTRAERNILESVKHPFIVELAYAFQTG 154	GKLYLILEYLSGGELFMQLEREGIFMEDTACFY 198 GKLYLILECLSGGELFTHLEREGIFLEDTACFY 187
- -	34	67 56	100	133	166 155
p70a.Prot.t p70b.Prot.t	p70a.Prot.t p70b.Prot.t	p70a.Prot.t p70b.Prot.t	p70a.Prot.t p70b.Prot.t	p70a.Prot.t p70b.Prot.t	p70a.Prot.t p70b.Prot.t

FIG. 2A-2

231	264	14/30 286 297	330	363 352	396 385
L A E I S M A L G H L H Q K G I I Y R D L K P E N I M L N H Q G H L A E I T L A L G H L H S Q G I I Y R D L K P E N I M L S S Q G H	VKLTDFGLCKESIHDGTVTHTFCGTIEYMAPEI KLTDFGLCKESIHEGAVTHTFCGTIEYMAPEI	L VRSGHNRAVDWWSLGALMYDMLTGAPPFTGENLVRSGHNRAVDWWSLGALMYDMLTGSPPFTAEN	RKKT I DKI LKCKL NLPPYLTQEARD LLKKLLKR RKKT MDKI I RGKLALPPYLTPDARDLVKKFLKR	NAASRLGAGPGDAGEVOAHPFFRHINWEELLAR NPSORIGGGPGDAADVORHPFFRHMNWDDLLAW	KVE RPFKPLLQS E ED VSQFDS KFTRQTPVDS PD RVD PPFRPCLQS E ED VSQFD TRFTRQTPVDS PD
199	232	265 254	298 287	331	364 353
p70a.Prot.t p70b.Prot.t	p70a.Prot.t p70b.Prot.t	p70a.Prot.t p70b.Prot.t	p70a.Prot.t p70b.Prot.t	p70a.Prot.t p70b.Prot.t	p70a.Prot.t p70b.Prot.t
		- · · 	· - · · · ·		

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F16. 2A-

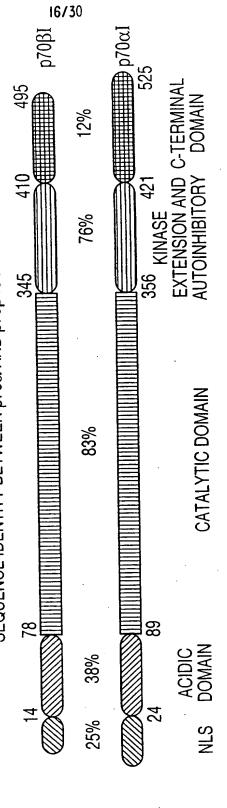
429	4 62	495	525
	15/3	474	495
DSTLSESANQVFLGFTYVAPSVLESVKEKFSFE	PKIRSPRRFIGSPRTPVSPVKFSPGDFWGRGAS	ASTANPOTPVEYPMETSGIEQMDVTTSGEASAP	L P I ROPNSGPYKKOAFPMISKRPEHLRMNL
DTALSESANQAFLGFTYVAPSVLDSIKEGFSFQ	PKLRSPRRLNSSPRVPVSPLKFSPFEGFRPS	PS - L - PE - PTELPL - PP - L L PPPPP - STTAP	L P I RPPSGTKKSKRGRGRPGR
397	430	463	496
386		450	475
p70a.Prot.t	p70a.Prot.t	p70a.Prot.t	p70a.Prot.t
p70b.Prot.t	p70b.Prot.t	p70b.Prot.t	p70b.Prot.t
	 .		

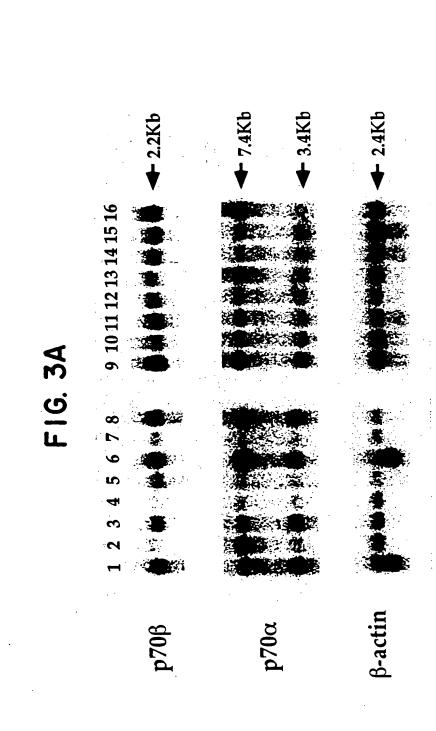
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APPLATE CS SUBCLAS

OSYMPHEM OSHOOL

FIG. 2B SEQUENCE IDENTITY BETWEEN $p70\alpha$ AND $p70\beta$ ISOFORMS

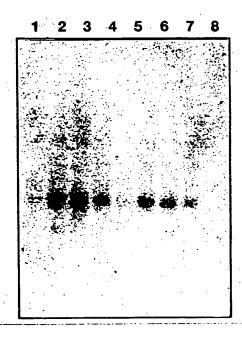




COVEEDS CSECL

FIG. 3B

Expression pattern of the p70 β mRNAs in tumour cell lines



- 1 Promyelocytic leukemia HL-60
- 2 HeLa cell S3
- 3 chronic myelogenous leukemia K562 4 Lymphoblastic leukemia MOLT-4
- 5 Burkitt's lymphoma Raji
- 6 colorectal adenocarcinoma SW480
- 7 Lung carcinoma A549
- 8 Melanoma G361

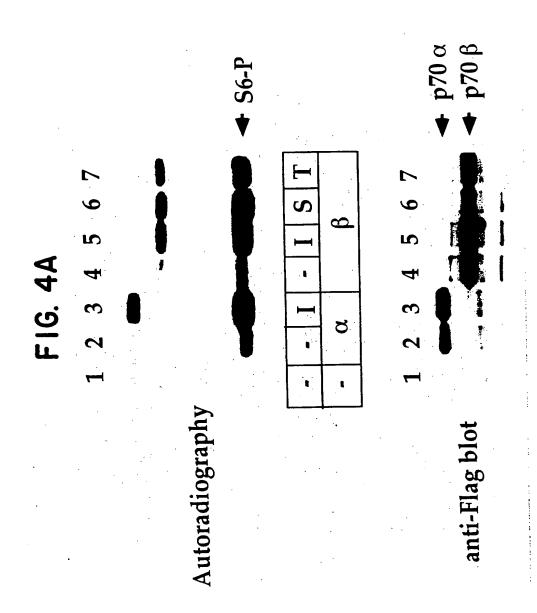
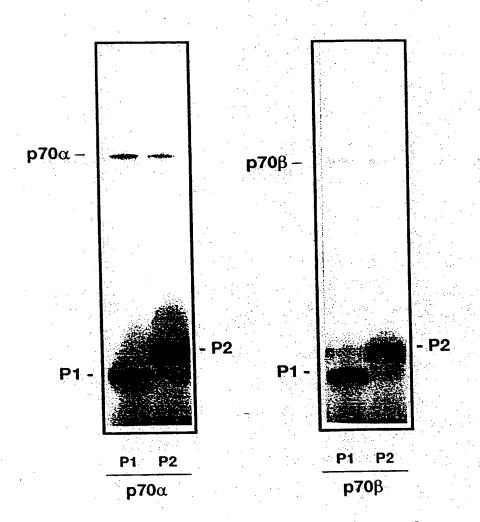
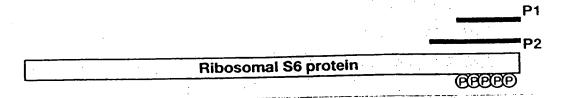


FIG. 4B

PHOSPHORYLATION OF THE RIBOSOMAL S6 PROTEIN C-TERMINAL PEPTIDES BY p70 α AND β KINASES



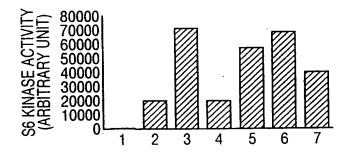


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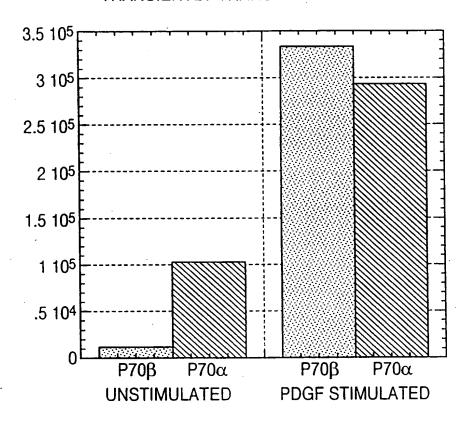
FIG. 5A

ACTIVATION OF THE P70 α AND β KINASES IN RESPONSE TO VARIOUS STIMULI IN VIVO



- 1 MOCK TRANSFECTION
- 2 p70α (STARVED AND NONTREATED)
- 3 p70α (STARVED AND INSULIN STIMULATED)
- 4 p70β (STARVED AND NONTREATED)
- 5 p70β (STARVED AND INSULIN STIMÚLATED)
- 6 p70β (STARVED AND SERUM STIMULATED)
- 7 p70B (STARVED AND TPA STIMULATED)





100 1000 200 8 0.2 Wortmannin(nM) Rapamycin (nM) Autoradiography anti-Flag blot

23/30

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FIG. 7

INTERACTION OF P70S6K β WITH DIFFERENT GST/SH3 FUSION PROTEINS IN VITRO

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

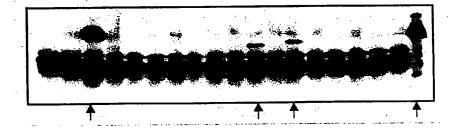




FIG. 8

IMMUNOPRECIPATATION AND WESTERN BLOT ANALYSIS OF P70βI AND βII TRANSIENTLY OVEREXPRESSED **IN HEK 293 CELLS**

Anti-p70β immunoprecipitation

Anti-p70β immunoprecipitation

48-

1 2 3 4

Anti-p70ß immunoblot

Anti-Flag immunoblot

1-mock transfection

2-Flag-p70αI

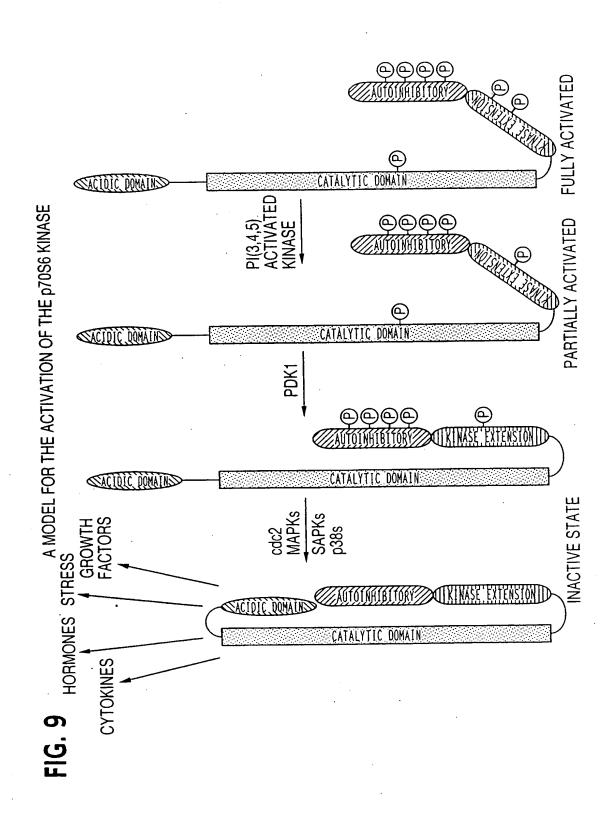
3-Flag-p70βI

4-Flag-p70βII

CLASS

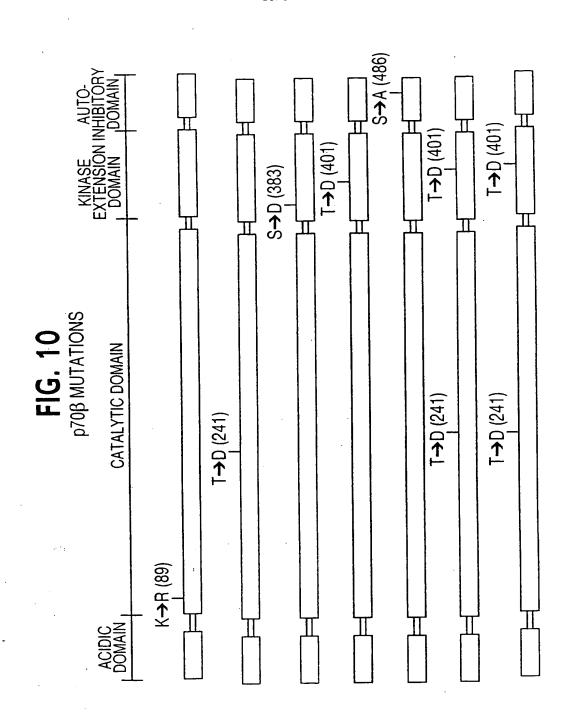
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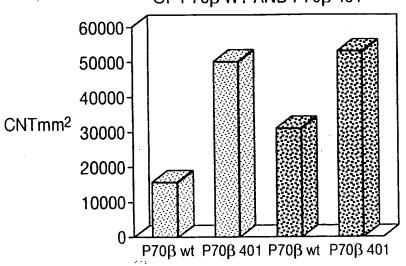


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S6 ACTIVITY

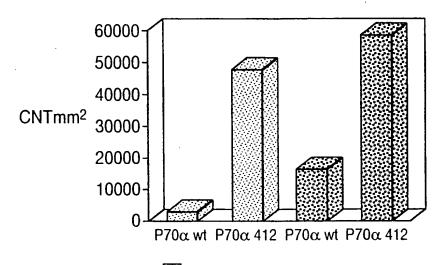
MAUTOPHOSPHORYLATION

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AFTSM

FIG. 12

COMPARISON BETWEEN THE ACTIVITY OF P70 α WT AND P70 α 412



S6 ACTIVITY

AUTOPHOSPHORYLATION